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Cankers of Cottonwood Cuttings in the Kansas State and Extension Forestry Nursery Rooting Beds: Fungal Associates and Control Evaluations

JULY 1980

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Cankers of Cottonwood Cuttings in the Kansas State and Extension Forestry Nursery Rooting Beds: Fungal Associates and Control Evaluations

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ABSTRACT

Evaluations were conducted to identify causes and attempt to control canker diseases on cottonwood cuttings after one growing season in rooting beds at the Kansas State and Extension Forestry Nursery in Manhattan. Isolations from cankered cuttings yielded a number of potential pathogenic fungi including *Phomopsis*, *Verticillium*, *Peyronellaea* (= *Phoma*), *Alternaria*, and *Epicoccum*. *Cytospora chrysosperma*, cause of cottonwood black stem, was not isolated from cankers; pathogenicity tests of associated fungi were not conducted. Soil fumigation of rooting beds and captan dip treatments of cuttings were tested to reduce canker losses. Neither treatment was effective in consistently increasing cutting survival and growth or reducing disease incidence. Cuttings from known-parentage stool beds had a greater survival rate than those from wild sources. Additional work is needed to evaluate pathogenicity of associated fungi and formulate more effective controls.

INTRODUCTION

Cottonwood rooting beds were established at the Kansas State and Extension Forestry nursery in Manhattan in 1977 to provide rooted cuttings for the statewide tree distribution program. First-year production of rooted cuttings was excellent. However, during 1978 canker diseases caused severe losses; only 25% of the planted cuttings developed into trees suitable for sale.

Previous investigations (11, 13, 23, 28, 30) have implicated *Cytospora chrysosperma* (Pers.) F., *Phomopsis* sp., *Fusarium* sp., and other fungi as causing cankers on and mortality of cottonwood cuttings in rooting beds. Because of the severe disease problem in Manhattan, evaluations were made to identify associated fungi and test the efficacy of soil fumigation and fungicide treatment in reducing losses.

MATERIALS AND METHODS

Soil fumigation was evaluated for effects on first-year survival, canker incidence and severity, and stem diameter growth of rooted cuttings. Twelve cottonwood selections were evaluated: six from known-parentage clones in stool beds and six from individual native cottonwood trees. The cuttings were collected in early March 1979, immersed in a captan [1/] solution (4.5 g Captan® 50 W per liter water), and stored for 3 months in sealed plastic bags at about 1° C. Cutting were approximately 15 cm long and 6-18 mm in diameter.

Rooting beds were amended with decaying wood chips and cow manure prior to fumigation. Soil pH averaged 7.1 at the time of fumigation; available phosphorus was about 1780 kg/ha. and exchangeable potassium was about 4660 kg/ha. Nitrogen at the rate of about 1940 kg/ha. was applied to beds prior to planting.

Treatment plots were fumigated with methyl bromide (137 kg/ha) three weeks prior to planting (May 1979). Cuttings were planted in a 5x15 cm spacing with 20 cuttings per row across beds. The twelve cottonwood sources were randomly distributed within fumigated and non-fumigated plots; there were three replications of each plot (120 cuttings per source per treatment).

1/ N - (Trichloromethylthio) - 4 - cyclohexene - 1, 2 - dicarboximide.

Pre- and post-fumigation soil samples were taken to determine effectiveness of methyl bromide in reducing standard soil pathogens. Samples were taken to a depth of approximately 15 cm within fumigated and non-fumigated plots; a total of 30 samples were taken. Each sample was cultured on selective media for presence of *Pythium* spp. (15) and *Fusarium* spp. (22). Estimates of soil populations were calculated as propagules per gram (ppg) of soil (field moist soil for *Pythium* spp., air-dried soil for *Fusarium* spp.).

Cankered cuttings were collected for isolation and identification of associated fungi. Wood chips from the edge of cankers was surface-sterilized in 10% sodium hypochlorite and transferred to water agar or potato dextrose agar (PDA) media amended with streptomycin (60 ppm). Cultures were incubated for 7-10 days at about 24° C. Hyphal tips of emerging fungi were transferred to PDA slants. Associated fungi were identified to genus using standard taxonomic guides (1, 20).

Effects of captan on first-year cutting survival, incidence and severity of cankers, and stem diameter growth were also evaluated. Three cottonwood selections, all from known-parentage stool beds, were evaluated. Half of the cuttings were immersed in fungicide solution and half in non-sterile tap water. Cuttings were planted as previously described in non-fumigated rooting beds. Three replications of each source and treatment resulted in evaluation of 720 cuttings.

All cuttings were irrigated overhead as needed during the growing season and evaluated for survival, disease incidence, and stem diameter growth in early December, 1979. Disease severity was based on extension of cankers around the circumference of cuttings. Those cuttings with cankers girdling greater than 50% of the circumference were classified as heavily diseased; those with smaller cankers were slightly diseased. Stem diameters were measured approximately 1 cm above the junction of stem and cutting.

Survival of cuttings within source groups (known-parentage stool stock and wild sources) was compared with two-way analyses of variance for both soil fumigation and fungicide application treatments. Paired t-tests were then used to compare total survival of treated and non-treated cuttings. Tukey's Multiple Comparison Test was used to locate sources which showed significantly different survival rates. Stem diameters of rooted cuttings were compared using one-way analysis of variance.

RESULTS

Cankers were mostly located laterally along cuttings (Figs. 1 and 2). They were numerous and girdled most of the circumference of heavily-diseased cuttings. Cuttings that did not survive may have been killed

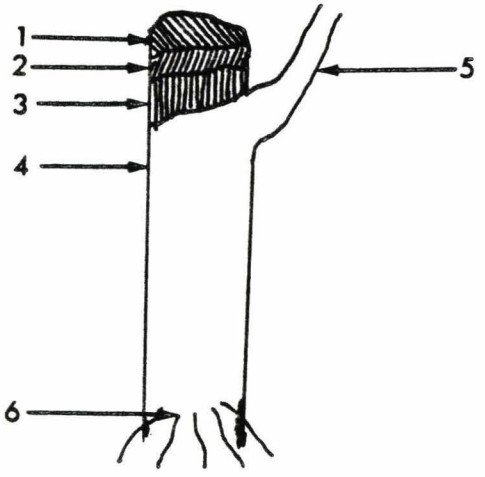
FIGURE 1. Cottonwood cutting with canker near the base.

FIGURE 2. A. Diagram of typical healthy cutting displaying necrotic zone at top.

1. Necrotic tissue: wood gray to light black, dry.
2. Necrotic tissue: wood brown. Zone of prominent sporulation by *Cytospora chrysosperma*.
3. Discolored tissue: wood orange to light brown, moist.
4. Non-discolored, healthy wood.
5. Stem of sprout (usually produced below the callus zone).
6. Rooting zone.

B. Diagram of typical lateral canker on cutting.

1. Non-discolored, healthy wood.
2. Discolored zone: wood orange to light brown.
3. Necrotic tissues: wood brown to black.
   Zone of sporulation by most associated fungi.



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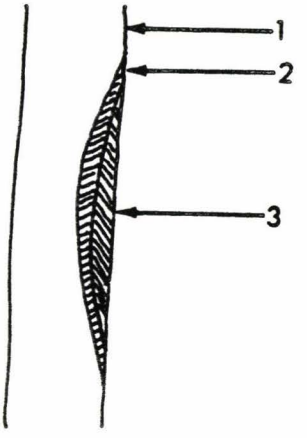

B

by canker fungi. Orange sapwood discoloration often bordered necrotic tissue (Fig. 2). Necrotic zones at the ends of cuttings were usually limited by host callus formation; stem extension occurred below this callus zone (Fig. 2).

Isolations made from the edge of cankers yielded many different fungi. Figures of the more commonly-isolated fungi are in the Appendix. *Cytospora chrysosperma*, the fungus most often associated with black-stem of cottonwood (13, 28), was not isolated from cankers. However, the fungus often sporulated within necrotic zones at the top of cuttings (Fig. 2).

Phomopsis sp. was frequently isolated from the edge of cankers; sporulation often occurred within necrotic tissues. Pycnidia of this fungus yielded both alpha and beta spores in large numbers (Fig. 3 A&B - Appendix). Other fungi commonly isolated from cankers included *Verticillium*, *Alternaria*, *Epicoccum*, *Penicillium*, *Mortierella*, *Curvularia*, *Coniothyrium*, and *Peyronellaea*. This latter fungus was placed in the genus *Peyronellaea* on the basis of chlamydospore structure (Fig. 3 D - Appendix). Pycnidial and conidial morphology were similar to *Phoma*. Boerema et. al. (6) consider *Peyronellaea* synonymous with *Phoma*.

Canker-causing potential of these fungi cannot be determined without properly conducted pathogenicity tests. More than one of these fungi may be involved. Non-identified bacteria, which were frequently isolated, may also be important.

Methyl bromide fumigation did not consistently increase survival nor decrease canker incidence and severity on rooted cuttings (Table 1). Analyses of variance indicated no significant differences ($P = 0.05$) in cutting survival between treatments for known-parentage sources; however, there was significantly greater survival in non-fumigated wild source beds. No significant differences in survival among sources (within each source group) were located with Tukey's Multiple Comparison Test (Table 1). However, cuttings from known-parentage sources had a much higher survival rate than wild cuttings from native cottonwood trees. No significant differences ($P = 0.05$) in disease incidence on surviving cuttings was detected. Stems of rooted cuttings were significantly larger ($P = 0.01$) in fumigated beds (Table 2). Pre- and post-fumigation counts of *Pythium* spp. and *Fusarium* spp. within rooting beds were inconclusive, indicating questionable effectiveness of the fumigation treatment in eliminating potential pathogens.

Captan treatment did not improve cutting survival within the three sources tested (Table 3); survival among sources was similar. Also, no significant differences in disease incidence on rooted cuttings was observed. Captan treatment also had no significant effect ($P = 0.05$) on stem diameter of live cuttings (Table 4).

TABLE 1. Effects of methyl bromide fumigation on one-year survival and canker incidence of cottonwood cuttings in rooting beds at the Kansas State and Extension Forestry Nursery.

Source	Fumigated				Non-Fumigated			
	No. of Live Cuttings			Percent Survival	No. of Live Cuttings			Percent Survival
	Total [1]	D_1 [2]	D_2 [3]		Total [1]	D_1 [2]	D_2 [3]	
Alton	76	11	13	63.3	62	2	13	51.7
Leavenworth 1	69	7	19	57.5	78	4	18	65.0
Leavenworth 3	68	3	14	56.7	81	3	11	67.5
Lydic	87	6	20	72.5	106	3	22	88.3
Ashford 1	90	8	18	75.0	97	0	12	80.8
Werner 2	54	4	8	45.0	62	4	14	51.7
Subtotal	444	39	92	61.7	486	16	90	67.5
Wild 1	12	2	4	10.0	25	1	4	20.8
Wild 2	6	2	3	5.0	10	1	2	8.3
Wild 3	3	1	1	2.5	15	1	4	12.5
Wild 4	0	0	0	0.0	2	0	0	1.7
Wild 5	25	5	4	20.8	43	4	6	35.8
Wild 6	19	3	6	15.8	27	2	5	22.5
Subtotal	65	13	18	9.0	122	9	21	16.9
Totals (all sources)	509	52	110	35.3	608	25	111	42.2

[1] Of a total of 120 cuttings per source. No significant differences (P = 0.05) among sources within known-percentage stool sources and wild sources were detected using Tukey's Multiple Comparison Test.

[2] D_1 = Diseased cuttings with more than 50% of their circumference girdled by cankers.

[3] D_2 = Diseased cuttings with less than 50% of their circumference girdled by cankers.

TABLE 2. Effects of methyl bromide fumigation on stem diameter of live cottonwood cuttings after one growing season.

Source	Fumigated		Non-Fumigated		F Value 1/
	No. Live Cuttings	Ave. Stem Diameter (mm)	No. Live Cuttings	Ave. Stem Diameter (mm)	
Alton	76	10.9	62	10.0	1.72 NS
Leavenworth 1	69	8.2	78	7.9	2.34 NS
Leavenworth 3	68	9.2	81	8.0	6.31 *
Lydic	87	8.6	106	7.7	4.69 *
Ashford 1	90	9.2	97	7.9	8.63 **
Werner 2	54	8.9	62	6.8	12.84 **
Wild Sources	65	5.2	122	6.3	6.89 *
Totals	509	8.6	608	7.5	30.80 **

1/ Based on one-way analysis of variance comparing cuttings from fumigated and non-fumigated rooting beds. NS = not significant (P = 0.05); ** significant at P = 0.01; * significant at P = 0.05.

TABLE 3. Effects of captan on one-year survival and canker disease incidence of cottonwood cuttings in rooting beds at the Kansas State and Extension Forestry Nursery.

Source	Captan Treated				Non-Treated			
	No. of Live Cuttings			Percent Survival	No. of Live Cuttings			Percent Survival
	Total 1/	D_1 2/	D_2 3/		Total 1/	D_1 2/	D_2 3/	
NE 20	43	2	4	35.8	52	3	5	43.3
NE 17	48	8	4	40.0	57	3	0	47.5
52 C	38	0	7	31.7	43	3	3	35.8
Totals	129	10	15	35.8	152	9	8	42.2

1/ Out of a total of 120 cuttings per source. No significant differences (P = 0.05) among sources were detected using Tukey's Multiple Comparison Test.

2/ Live diseased cuttings with more than 50% of their circumference girdled by cankers.

3/ Live diseased cuttings with less than 50% of their circumference girdled by cankers.

TABLE 4. Effects of captan immersion on stem diameter of live cottonwood cuttings after one growing season.

Source	Captan Treated		Non-Treated		F Value 1/
	No. Live Cuttings	Ave. Stem Diameter (mm)	No. Live Cuttings	Ave. Stem Diameter (mm)	
NE 20	43	8.2	52	7.7	1.71 NS
NE 17	48	7.9	57	8.2	1.01 NS
52 C	38	9.7	43	9.1	0.99 NS
Totals	129	8.5	152	8.3	0.81 NS

1/ Based on one-way analysis of variance comparing cuttings that were captan treated and non-treated. NS = not significant (P = 0.05).

DISCUSSION

Because of its common association with cankers of cottonwood cuttings (11, 16, 28), we expected to isolate *C. chrysosperma* from diseased cuttings. Although the fungus was common on necrotic tissues at the exposed top of cuttings, it was not associated with cankers. *Cytospora* is often saprophytic on different substrates (20, 26). However, it may infect live tissues through wounds (24) or during periods of host stress (3, 4, 8, 24). Moisture-stressed trees are especially susceptible (4). These trees were not under stress, and therefore probably not susceptible to *C. chrysosperma*.

Phomopsis sp. was commonly associated with cankers. These fungi commonly cause cankers on woody plants. For example, *P. cinerescens* (Sacc.) Trav. causes cankers of figs (*Ficus* spp.) in California and Europe (16, 21). Also, *P. conorum* (Sacc.) Died. incites cankers on white fir in Iowa (12), and *P. lokoyae* Hahn. is associated with Douglas-fir cankers in California (7). *Phomopsis* has also been associated with blackstem of cottonwood cuttings (23, 28). Pathogenicity of *P. macrospora* Kubayashi & Chiba was confirmed on rooted cuttings (11); the fungus caused more cankers, but less mortality than *C. chrysosperma* on inoculated cuttings. *Phomopsis macrospora* has also been associated with cankers of plantation-grown cottonwoods in Mississippi (10). Therefore, *Phomopsis* may be important in causing cankers on cottonwood cuttings.

Another fungus commonly associated with cankers was *Verticillium* sp. Although most pathogenic forms of this fungus cause vascular wilts (9, 25), some may be associated with cankers. Some species of *Verticillium* have *Nectria* spp. as an imperfect stage, known canker organisms on branches and small stems of hardwood trees (2, 26). However, *Nectria* was not found on the cankers we evaluated nor was it produced in *Verticillium* cultures obtained from these cankers. Therefore, the role of *Verticillium* in this disease is questionable.

A pycnidial-forming fungus classified as either *Peyronellaea* or *Phoma* was commonly isolated from cankers. *Peyronellaea* has been associated with cottonwood blackstem (13), although it was not pathogenic. *Phoma* has been associated with lesion-type diseases of crucifers (27) and lodgepole pine seedling mortality (18). Both fungi are soil inhabitants (13, 18); their pathogenic role may be secondary.

Isolations from cankers often yielded *Alternaria* sp. Members of this genus are common soil inhabitants, saprophytic on organic debris (2). However, *Alternaria* may be associated with necrosis of leaf and stem tissues (26, 27). *Alternaria* was previously isolated from advancing canker margins of cottonwood nursery stock (13). However, these isolates were not pathogenic. *Alternaria* was probably a secondary invader of cankers.

Another fungus often sporulating on necrotic canker tissues was *Epicoccum* sp. These organisms are mostly saprophytic, but sometimes weakly parasitic (26). *Epicoccum* is common on many different plants, decaying herbaceous debris and wood, and commonly isolated from soil (1, 26). Non-pathogenic strains of *Epicoccum* have been associated with cankers of cottonwood nursery stock (13). This fungus was probably a secondary colonizer of cottonwood cuttings.

Other common soil fungi isolated from necrotic canker tissues include *Mortierella, Penicillium, Gonatobotrys, Curvularia,* and *Coniothyrium.* All are mostly saprophytic, although *Penicillium* rots conifer seeds (17) and *Curvularia* and *Coniothyrium* are associated with leaf spots (26) and rose stem necrosis (1), respectively.

No significant treatment effect on survival and disease incidence was observed for soil fumigation. Ineffectiveness of soil fumigation may indicate that: (1) treatments did not sufficiently lower soil pathogen populations, (2) pathogens inciting cankers were not soil-borne, (3) pathogens rapidly reinvaded fumigated soil from surrounding non-fumigated beds, or (4) pathogens contaminated cuttings before planting and were not removed by or reinvaded tissues after fungicide treatment.

Most organisms isolated from diseased cuttings were soil residents. The major exception, *C. chrysosperma* (8), was not associated with cankers. Therefore, we expected fumigation to decrease disease by destroying canker fungi. Because treatment blocks in rooting beds were small, there was high probability for reinvasion of fumigated soil by pathogens. Reinvasion would be especially rapid if pathogen populations in surrounding soil were high. Because of the inconclusive results of pre- and post-fumigation counts of standard soil pathogens, we question effectiveness of the treatment. Our results indicated that methyl bromide may not have effectively reduced canker fungal populations sufficiently to reduce disease.

Many fungi associated with cankers were facultative parasites; soil fumigation might have indirectly enhanced parasitism by these organisms. Under normal conditions, pathogenic behavior of soil-borne fungi is mediated by rhizosphere microflora (19). Removal of rhizosphere populations by fumigation may have enhanced pathogenicity of rapidly-invading pathogens by eliminating substrate competition.

If canker fungi contaminated cuttings at the time of planting, they would be expected to proliferate in the "biological void" created by fumigation. Without competing soil fungi and bacteria, these pathogens may incite disease readily.

Larger diameter, more vigorous cuttings generally produce large stems (14). Since cutting diameters were not measured and correlated with subsequent stem diameter, the larger diameters found in fumigated beds may not reflect treatment effects. However, removal of facultative parasites may account for improved vigor of surviving cuttings in fumigated beds.

Considering previous reports (13, 28), we expected captan treatment to increase survival and growth, and decrease canker incidence on cuttings. However, soil levels of pathogens may have been so high that fungicide effects were not sufficient to protect cuttings. Residual effects of the fungicide may also have deteriorated during storage time between treatment and planting.

Responses of different sources to diseases should be considered in any tree improvement program. We found large differences in performance between known-parentage sources and wild sources from native cottonwood trees. However, no single source was superior to others in survival, resistance to disease, or stem diameter growth.

Additional work is required to more fully elucidate etiology of cankers. Pathogenicity of associated fungi and responses of different sources to these organisms should be tested. Additional control trials are needed to evaluate efficacy of other chemical and cultural methods of improving survival and growth and reducing diseases on cuttings.

This publication reports evaluations involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife -- if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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A P P E N D I X

Figure 3.

A. *Phomopsis* sp. Hymenium bearing conidia with sporogenous cells mixed with sterile paraphyses.

B. *Phomopsis* sp. Alpha (x) conidia (arrow 1); beta (B) conidia (arrow 2).

C. *Peyronellaea* sp. Pycnidium.

C. *Peyronellaea* sp. Chlamydospores (arrows).

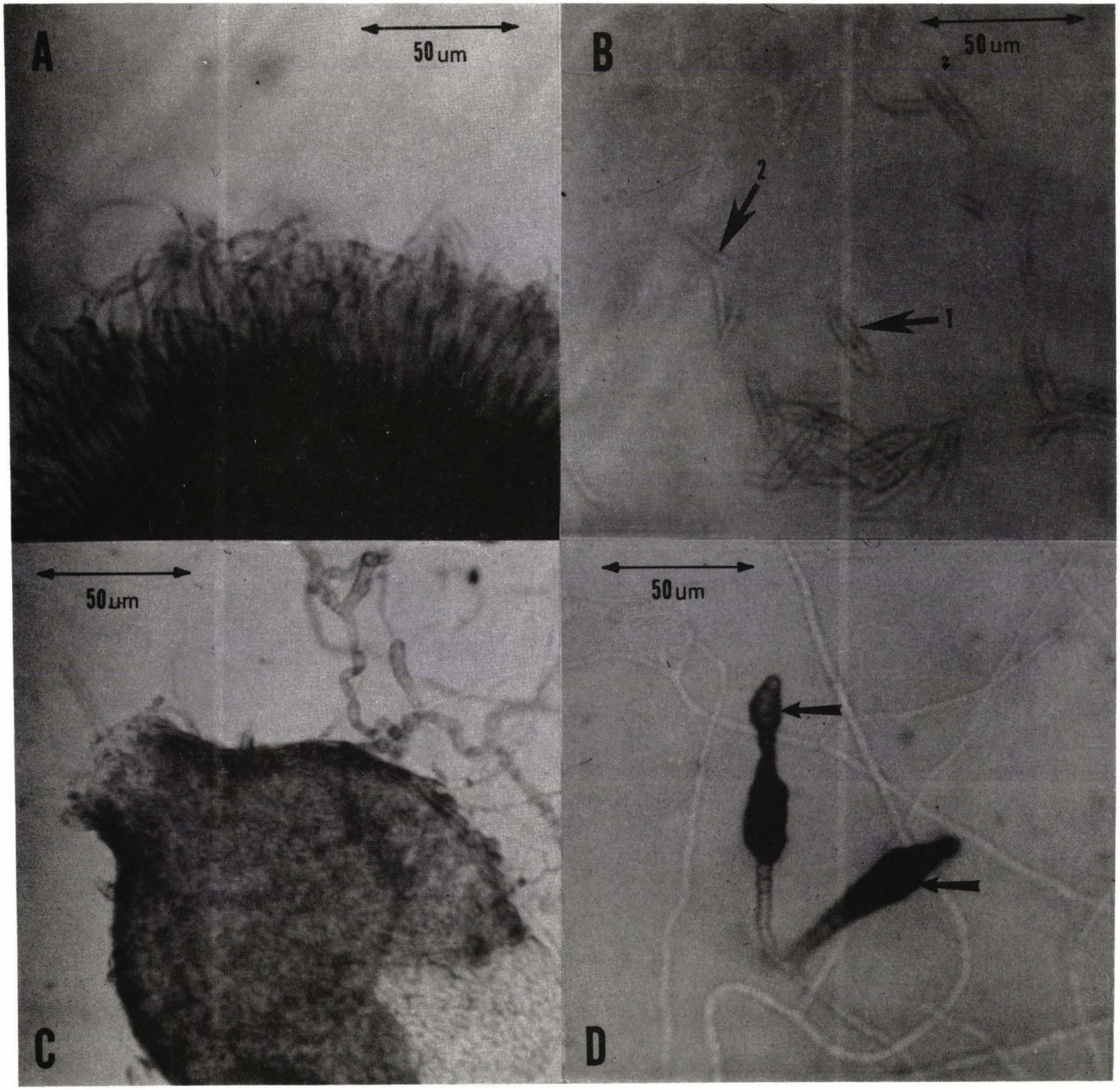

FIGURE 3.

Figure 4.

A. *Alternaria* sp. Chain of multi-septate conidia.

B. *Epicoccum* sp. Sporodochia.

C. *Gonatobotrys* sp. Conidiophore with intercalary, inflated, denticulate cells bearing conidia (arrow 1). Conidia borne singly on teeth (arrow 2).

D. *Curvularia* sp. Conidiophores (arrow 1) bearing curved, multi-celled, lightly pigmented conidia (arrow 2).

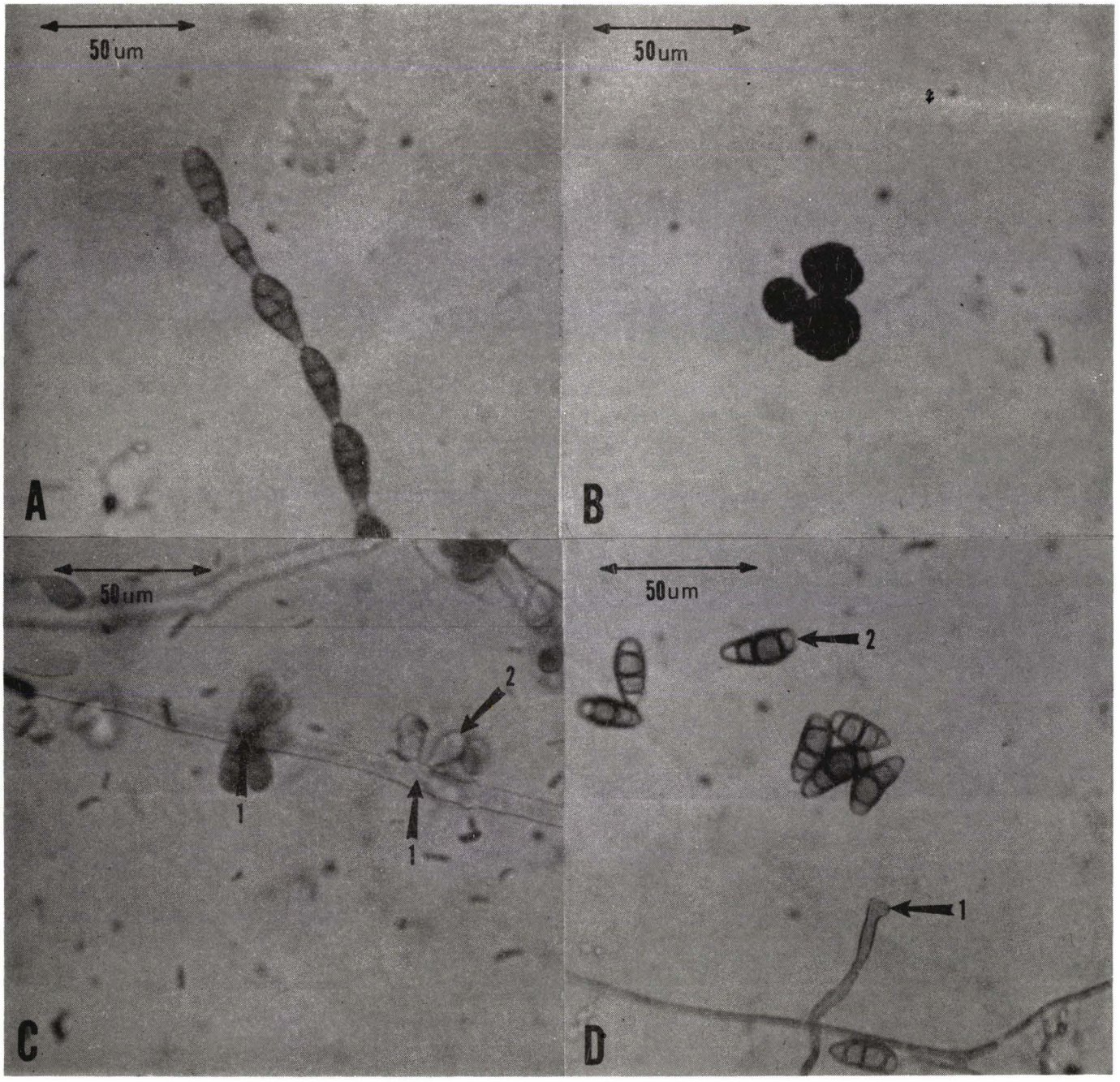

FIGURE 4.